The large-scale logico-chemical structure of a transcriptional regulation network

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Identity, response to external stimuli, and spatial architecture of a living system are central topics of molecular biology. Presently, they are largely seen as a result of the interplay between a gene repertoire and the regulatory machinery of the cell. At the transcriptional level, the *cis*-regulatory regions establish sets of interdependencies between transcription factors and genes, including other transcription factors. These "transcription networks" are too large to be approached globally with a detailed dynamical model. In this paper, we describe an approach to this problem that focuses solely on the *compatibility* between gene expression patterns and signal integration functions, discussing calculations carried on the simplest, Boolean, realization of the model, and a first application to experimental data sets .

I. INTRODUCTION

Regulation can be defined as the set of physico-chemical constraints operating within a living cell that modulate the expression of the cell's genes. In the present view of molecular biology, regulatory processes are often used as a primary causal explanation for many phenomena, playing a role in this discipline that is comparable to the role fundamental interactions play in physics. In

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fact, it is widely believed that the repertoire of signal responses (and, more in general, of all the information processing and structural tasks) of living systems is encoded in interconnected threads of genes regulating the activity of each other. These networks of interdependencies are still largely uncharacterized, although they have begun to fall within reach of systematic experimentation in the recent years (Babu et al., 2004; Herrgard et al., 2004; Lee et al., 2002; Nachman et al., 2004; Wolf and Arkin, 2003).

Considering the so-called “central dogma” of molecular biology,

$$\text{DNA} \overset{\text{transcription}}{\longrightarrow} \text{mRNA} \overset{\text{traslation}}{\longrightarrow} \text{protein} \overset{\text{folding}}{\longrightarrow} \text{function},$$

regulation processes can intervene at all the separate steps (and also in different sub-steps). Regulation exploiting the process of transcription, or transcriptional regulation, constitute to date the best understood among all the possible regulation mechanisms.

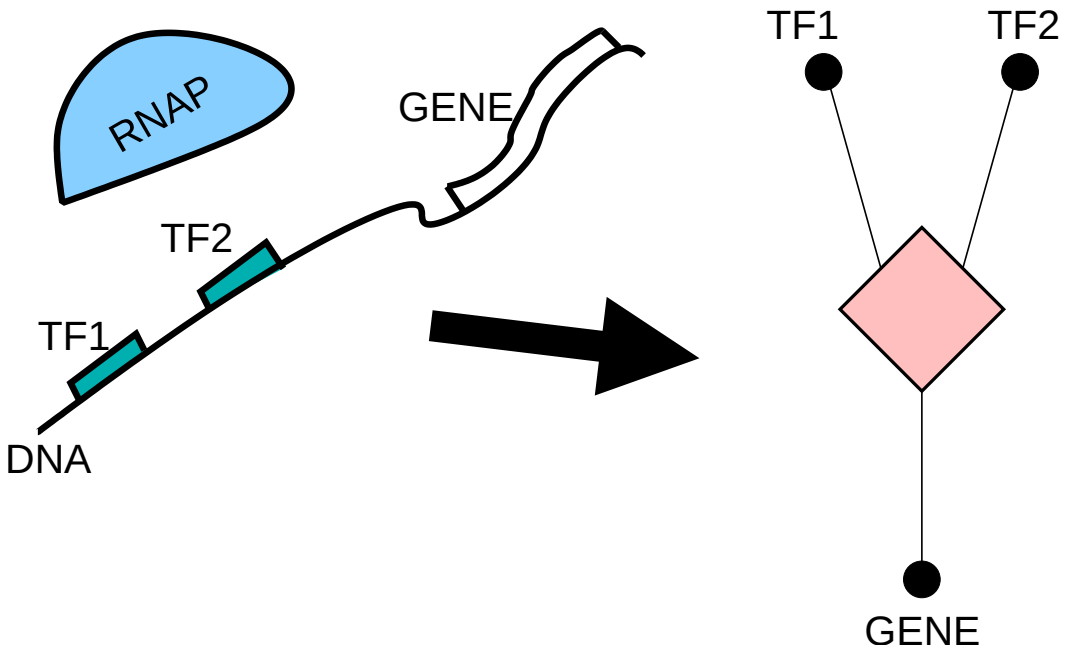


FIG. 1 Schematics of our representation of a signal integration function at the *cis*-regulatory region of a gene as a constraint on the gene expression variables. For general variables, the constraint involves minimization of the free energy of the Shea-Ackers model. In GR1, the constraint is Boolean.

Transcriptional regulation networks are defined starting from the basic functional building blocks involved in transcription. These are (i) the promoter region of a gene or operon along the DNA sequence, which contains the *cis* regulatory binding sites for the transcription factors, (ii) the transcription factors, which are proteins that regulate the binding of RNA-polymerase, and

(iii) RNA-polymerase, the protein complex that performs transcription of a gene or an operon in mRNA form (Alberts et al., 2003; Ptashne, 1992). The amount of mRNA transcribed is related to the expression of a particular gene only if one takes for granted all the other steps that bring to a functional protein. If this (big) leap is accepted, the "state" of a cell is identified to the mRNA concentration of its genes. Experimentally, this is particularly sound for prokaryotes and simple unicellular organisms, but often assumed in more complex contexts, for example in DNA microarray experiments. Under this assumption, the locations and orientations of the binding sites for transcription factors, as well as the affinity of the transcription factors to different binding sites, determine the expression levels of a gene in response to changes in the active transcription factor concentrations inside the cell. In turn, the concentration of active transcription factors (the ones that can actually bind) encodes the configuration of the environment, for example through degradation or activation by internal and external signaling molecules. A *cis*-regulatory region can contain many binding sites for many transcription factors which act in cooperation (or competition) on the promoter region, to control in a combinatorial way the binding of RNA polymerase. This process, referred to as signal integration, is the logic heart of the network. A transcriptional regulation network can be represented as a hypergraph containing both gene expression ("variable") nodes and signal integration ("function") nodes. The connectivity is the source of the network complexity (Fig. 2).

Thus, a transcriptional regulation network can work independently as a computational unit in a living cell, being able to make decisions on which genes will be switched on at different times. Studies focusing simply on the *structure* of the underlying graph have lead to interesting results (Babu et al., 2004; Shen-Orr et al., 2002; Warren and ten Wolde, 2004). However, characterizing and predicting gene expression patterns given a network structure remains an enormous challenge. Two main problems exist. Firstly, the networks are only partially characterized experimentally (Herrgard et al., 2004; Nachman et al., 2004). In some instances (Babu et al., 2004; Davidson et al., 2002), the wiring diagram is well known, but in general the functions are described only qualitatively, typically with annotations such as activation, repression or dual effects, and little is known about their actual structure. Secondly, transcription networks are fairly large. While detailed models or simulations work well on small (sub-)systems (Arkin et al., 1998; McAdams and Arkin, 1997), typically a coarse grained approach is needed. Microscopically, it is well accepted that the Gillespie algorithm (Gillespie, 1977), while disregarding spatial correlations, correctly describes the stochastic asynchronous events of chemical kinetics involved. On

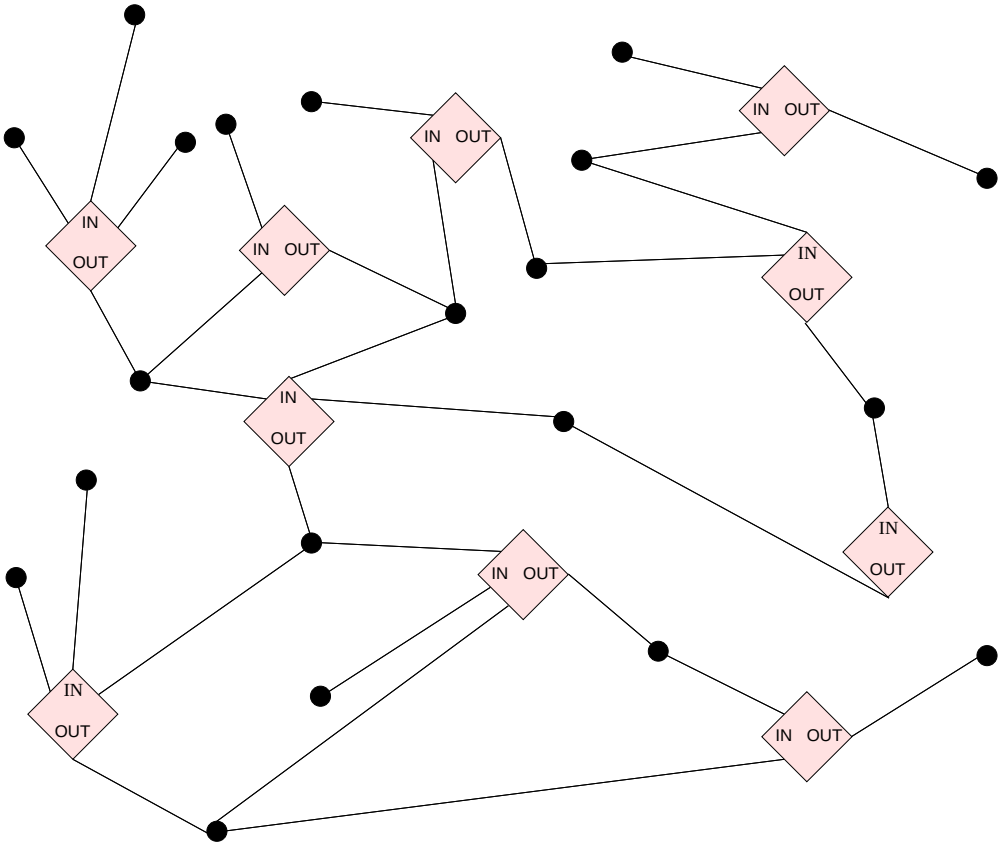


FIG. 2 Graph representation of a transcription network. Each diamond node represents a signal integration function, while each black circle is a variable. The directionality of the constraint is represented graphically by labeling the diamonds with IN and OUT on two different sides.

the other hand, with a mesoscopic average in time, it is still unclear what the emergent time scales might be. In particular, the pioneering approach of Kauffman (Kauffman, 1969a,b, 1993, 2004), suggesting a synchronous deterministic dynamics for a Boolean (i.e. ON/OFF) representation of the network is still being debated, both in its assumptions and in its results (Bastolla and Parisi, 1998; Gershenson, 2004; Kaufman and Drossel, 2005; Socolar and and Kauffman, 2003).

We consider the second problem, and develop a model (called GR, from Gene Regulation), that focuses, rather than on pure dynamics, on the compatibility between gene expression patterns and signal integration functions. The compatibility constraints are generated by the clauses encoded by signal integration functions at *cis*-regulatory regions. Our framework describes the system as a combinatorial optimization problem where N variables, the gene expression levels, are subject to M constraints, representing the signal integration nodes. Simply put, a cell with N genes can express them in exponentially many ways, 2^N in the Boolean ON/OFF representation. However, the cell never explores all the possible patterns of expression. It generates only clusters of corre-

lated configurations. To fix the ideas, one can think to the very elementary example of the cI-cro switch of λ-phage (Ptashne, 1992; Thomas, 1973). In this case one could observe the states 10 (where cI is ON, and cro is OFF), 01, or perhaps 00, but never 11, because this state is ruled out by the signal integration function. In a cell, with the added complexity of the regulation network, we can think that many of the states are not observable for the same compatibility reasons. The approach is easily connected to detailed thermodynamic treatment of transcription from a signal integration node on one side (Buchler et al., 2003), and to the statistical mechanics of spin glasses and combinatorial optimization problems on the other (Mezard et al., 2002).

Rather than the detailed quantitative prediction of mRNA expression states, the current challenge is to set a conceptual framework which can help to interpret the observations in concrete examples, integrating as much as possible with known data. As a the simplest example of this, we study the behavior of the Boolean version of our model on the network structure of E. coli. Building up from this simplest case, the aim is to analyze increasingly realistic network structures, in order to generate a theory that, while being consistent with the generic qualitative features of regulation networks, is useful to analyze single instances and realizations. This is maximally important as biological knowledge is constructed on specificities, and not on typical case behavior.

This paper is structured as follows. Sec. II introduces the model abstractly, as an optimization problem, which, in sec. III is connected to the more concrete thermodynamic Shea-Ackers model of transcriptional regulation. Sec. IV abandons this general setting, and takes on the simplest possible formulation of the model, GR1, which has Boolean functions and variables, showing that this case maps directly to a so-called satisfiability problem (Sat). The scope of sec. V, is to analyze the typical number $\mathcal{N}$ of gene patterns of a random instances of GR1, starting from the case of fixed connectivity. The "leaf removal" algorithm allows to carry this analysis in the annealed approximation. An important premise is the fact, which is evident looking at the data (Shen-Orr et al., 2002), that some genes are essentially "free" from the point of view of transcription. These are mainly controllers and are connected to external stimuli. The expression of the rest is conditioned to the state of other genes. The algorithm allows to define the "complex combinatorial core" (CCC) of the network, as the set of genes able to control its global state. The number of non-controlled, or "free" variables in the core determines the complexity of the system. The phase diagram shows three distinct regimes of gene control. In the first (UNSAT), there are no free genes in the core, and the system cannot control the simultaneous expression of all its genes. In the second regime ("complex control" or HARD-SAT), the core contains free genes that control, both directly and

indirectly, many others. The general dynamics is residual (many variables are fixed, the others can change). In the third regime, the core is empty. Each free gene (which is external to the core) controls the state of a small number of genes ("simple control", or SAT phase). Sec. VI concerns itself with the *width* of the distribution of $\mathcal{N}$, which has both a technical significance as a validity test of the annealed approximation, and a biological one, as the variability in the number of gene patterns at fixed gene number. Sec. VII discusses generalizations of these results to non-fixed connectivities. Finally, sec. VIII describes one first attempt to put this findings to work on an experimental data set.

II. MODEL

Our aim is to describe in a minimal way gene expression in a transcription network, separating the issues related to the dynamics from those related to its logical and computational structure. In order to do this, we will formulate a model that sees the system as an optimization problem, where a set of variables, the genes, is subject to a set of constraints, the signal integration nodes. Upon this logic backbone, many a dynamics can be superimposed, including in the most general case the kinetic Montecarlo scheme commonly used to model genetic networks. Rather than going towards the direction of highest detail, we will choose to simplify the model as much as possible, reducing the number of details to the minimum, and studying the general qualitative features of the system.

The model is specified by

1) A set of N discrete variables $\{x_i\}_{i=1..N}$ associated to genes or operons, which in the simplest picture are identified with their transcripts and protein products. These variables represent the expression levels and in general take discrete values in $\{0, .., q\}$. In particular situations, they are well-approximated by continuous variables.

2) A set of M interactions, or constraints $\{I_b(x_{i_0}, x_{i_1}, ..., x_{i_{k_b}})\}_{b=1..M}$ between the genes, representing the signal integration from transcription nodes.

This formulates an optimization problem, which we call GR, from Gene Regulation. GR asks to find the states compatible with the constraints.

The model can be easily generalized to include other relevant degrees of freedom, such as translation, protein modification and protein-protein interactions. However, each addition adds complexity and parameters. Therefore we start with the minimal possible description. Admittedly,

neglecting non-transcriptional regulation is a drastic simplification of the system. A complete genetic network should in principle include all forms. On the other hand, the justification for considering transcription alone is that it is the first step in the chain of regulation events and it is experimentally well characterized. From the physics point of view, this model can be seen as a "spin glass", a system where some variables, our gene expression levels, interact through some coupling constants, specified by the constraints (Mertens, 2002). This approach to optimization problems of computer science has proved to be very useful in the recent years (Mezard et al., 2002).

The network structure is naturally represented on a "factor graph", where two kinds of nodes are present, N "variable nodes" and M "function nodes" respectively (Fig. 2). Here $k_b = 1 + K_b$ can be seen as the local connectivity of a function node. Note that the factor graph is also defined by a variable connectivity c_i, the number of functions connected to x_i. In fact, this is the typical structure of a constraint satisfaction problem of theoretical computer science, such as q-coloring or satisfiability (Mertens, 2002).

III. THE CONSTRAINTS AND THE SHEA-ACKERS MODEL

To specify the model one has to give a structure for the function nodes, i.e. the constraints. This requires a physical model for signal integration. In order to take this step, in this section we start from the well-known and widely accepted thermodynamic model of Shea and Ackers of gene activation by recruitment, to show that it is the natural setting to express our constraints.

Let us consider a function node with K regulators and one output variable. This is modeled, in the version presented by Buchler and collaborators (Buchler et al., 2003), as a neural network (a "Boltzmann machine") with Hamiltonian

$$H = \sum_{\substack{i,j=0..L \\ i \neq j}} J_{ij} s_i s_j + \sum_{j=0..L} h_j s_j \ ,$$

where $s_1, .., s_L$ are the occupation variables of the *cis*- binding sites, h_j are external fields, functions of $x_1, ..., x_K$ representing the concentrations of "input" transcription factors, and J_{ij} are interaction constants associated to competitive versus cooperative binding. More precisely, $h_j = -\beta^{-1} \log(Q_j)$, where $Q_j = \frac{[TF_i]}{\kappa_i} \sim \frac{x_i}{\kappa_i}$ is the binding affinity of a site i, and κ_i a dissociation constant. Normally, the concentrations are approximated with continuous variables. In general, $L \geq K$, because multiple binding sites are present. Finally, s_0, h_0 are the occupation variable and

the external field (a fixed parameter corresponding to the polymerase binding affinity) associated to the output node of the function (see Fig 3).

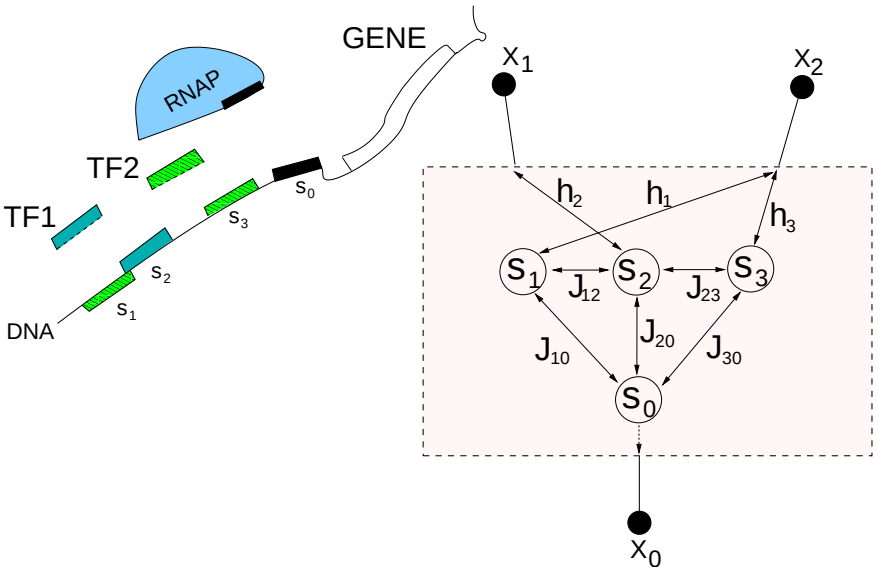


FIG. 3 Exemplification of a Shea-Ackers node. s_i are occupation variables for the transcription factors binding sites, while the coupling constants J_{ij} encode cooperative or competitive binding. The external fields h_i are the phase space variables of GR.

Given all the binding constants and the interaction parameters, the -intrinsically probabilistic- output of the gate is computed as a function of the input fields, simply as the probability that $s_0 = 1$. This expectation value can be obtained through the partition function

$$Z[h_1, ..., h_L] = \sum_{\{s\}} e^{-\beta H}$$

as

$$P(\sigma_0 = 1) = \frac{1}{Z} \sum_{\{s\}} e^{-\beta H(1, s_1, ..., s_L)}$$

where $\beta = 1/kT$.

Equivalently, one can compute the local free energy

$$-\frac{1}{\beta} \log Z = F[h_0, ..., h_L] = F[x_0, ..., x_K] \tag{1}$$

and find the average output through minimization with respect to the x_0 coordinate.

In other words, the function nodes are local equilibrium conditions for the variable nodes, specified by the Shea-Ackers model of the *cis*-regulatory region of each variable node. The expression variables $\{x_i\}_{i=1..N}$ need to satisfy the constraints specified by the local minimizations

of the free energies $\{F_b(x_{i_0}, x_{i_1}, ..., x_{i_{k_b}})\}_{b=1..M}$. Since there is a clear input-output logic encoded by the chemical equilibrium of each signal integration nodes, one could refer to this backbone static structure the "logico-chemical" structure of the network, and separate it from its "dynamic" structure. The logic it encodes is of course not Boolean. In fact, it is intrinsically non-Boolean even with Boolean variables, as the outputs are probabilistic functions of the inputs.

From the point of view of statistical mechanics, this is a Potts spin system with diluted interactions described by the local free energies F_b (which in this context should be interpreted as effective Hamiltonians). Interestingly for the analogy with spin glasses, the model for the gate can be seen as a message-passing procedure analogous to that exploited by the cavity method (Mezard and Parisi, 2003; Mezard et al., 1987; Mezard and Zecchina, 2002), where, in the approximation of factorized probability distribution of the variables, one evaluates the local fields $h_{i\to b}$, describing the local influence of the couplings on variable i in absence of interaction b, and $u_{b\to i}$, the contribution of interaction b on the local magnetic field on spin i, together with their histograms in the presence of many states. In our case, the "messages" described above can only travel in the input-to-output direction. We are currently investigating whether this analogy can be exploited for further calculations, and we are aware of work in this direction, in a simpler setting, by another group (Correale et al., 2004).

In principle, the variables x_i directly stand for the number expressed of molecules in a cell. Provided the set of all binding constants and interactions is known, all the function nodes can be computed and the model is complete. It could be solved, for example by numerical simulations, once a dynamics is specified.

On the other hand, with a few exceptions of small systems, these (many) parameters are in general not known. For this reason, rather than aiming for the highest level of detail, we choose to simplify as much as possible, while trying to keep the most relevant features. For practical purposes, in order to be able to advance further analytically and numerically in the understanding of the model, it is convenient to introduce coarse grained expression levels, thereby effectively reducing q. The resulting model, GRq, is identical, mind the fact that variables and constraints are now subject to implicit averaging. One advantage of this approach is that local free energies become easier and easier to specify, and it is possible to study, as is commonly done for spin glasses, the typical behavior of the system as a function of the parameters.

In the simplest possible scenario $q = 1$, and the expression levels are Boolean variables. The assumption behind this is that what matters is only if the level of expression is high or

low (Kauffman, 1969b, 1993). The simplest case (which we will still call GR1), assumes also Boolean functions. In the following section, we will show how GR1 maps to a Satisfiability problem (Sat), an optimization problem where N Boolean variables are constrained by M conjunctive normal form (CNF) constraints (i.e. by a Boolean polynomial constructed as a product ($\wedge$) of M disjunctive monomials ($\vee$)).

IV. GR1, MAPPING ON A SATISFIABILITY PROBLEM

As we have shown in the above section, for general variables x_i that represent real expression levels, the constraints can be derived directly from the model of Shea and Ackers of gene activation by recruitment (Buchler et al., 2003; Shea and Ackers, 1985). If the x_i represent coarse-grained expression levels, the same model can be used to construct the local free energy in Eq. (1), associated to each signal integration node, that generates the constraints through minimization. Here we consider the simplest possible scenario, treating the expression levels as Boolean variables, setting $q = 1$, and the signal integration functions as boolean functions $\{f_b(x_{b_1}, .., x_{b_{k_b}})\}_{b=1..M}$. We also restrict to the case of fixed in-ward connectivity $k_b = K, \ \forall b$. These conditions, defining K-GR1, are also found in Kauffman networks (Gershenson, 2004; Kauffman, 1969b). We will relax the hypothesis of fixed K to explore networks with fluctuating connectivity in section VII.

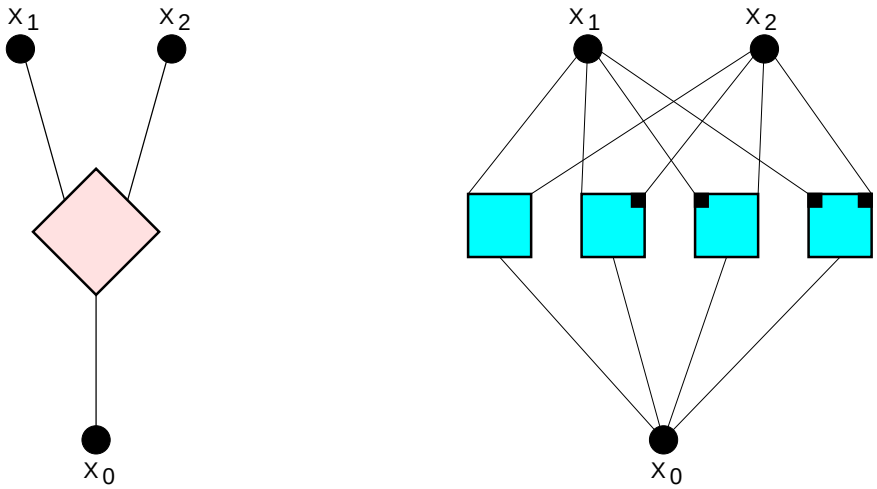


FIG. 4 Translation of a 2-GR1 node in 3-Sat Nodes. The input-output direction is from top to bottom. Sat constraints are represented as squares, where the black and white vertices indicate that the corresponding variable enters negated or affirmed respectively in the 3-Sat constraint.

The expression $x_{b_0} = f_b(x_{i_1}, .., x_{i_K})$, which imposes that the variable x_{b_0} is the output of the

function f_b, translates into the Boolean constraint

$$\neg(x_{b_0} \dot{\vee} f_b). \tag{2}$$

In a Kauffman network, this expression is equivalent to the fixed point condition, and there is one such constraint for every variable x_i. The formula

$$I = \bigwedge_{b=1..M} I_b = \bigwedge_{b=1..M} \neg(x_{b_0} \dot{\vee} f_b)$$

defines a Satisfiability problem (Sat) on the variables $x_1, .., x_N$. From the biological viewpoint, this is a logic representation of the computational tasks encoded in the transcription network by evolution, i.e. which sets of genes have to be switched on at any given condition. More abstractly, having in mind Kauffman networks, each satisfying solution of this problem corresponds to a fixed point in the Kauffman dynamics (independently from the update scheme). The number of variables involved in one constraint is always exactly $k = K+1$, therefore this mapping associates a network with fixed connectivity K, to a k-Satisfiability problem whose connectivity is increased by one unit. For example, a $K = 2$ Kauffman network corresponds to a 3-Sat problem, and so on. The suitable order parameter for such a system is $\gamma = M/N$. GR1 assumes that each gene expression variable is regulated at most by one signal integration function, so that $\gamma \leq 1$.

To further understand the logic structure if GR1, we can write the CNF constraints on each variable x_n. This allows to make a connection to the order parameters used in k-Sat, i.e. the local constraint α, defined as the number of conjunctive-normal-form (CNF) clauses per Boolean variable. In order to do this, we recast the Boolean formulas I_n into CNF. Reshuffling the truth table of f_n in a way that the first z terms $(1, .., z)$ give zero as an output, a simple procedure shows that

$$\begin{aligned} I_b \; = \quad & \Big(\textstyle\bigwedge_{\alpha=1}^{z} (\neg x_{b_0} \vee \xi_{\alpha_1} \vee .. \vee \xi_{\alpha_K}) \Big) \\ & \bigwedge \Big(\textstyle\bigwedge_{\alpha=z+1}^{2^K} (x_{b_0} \vee \xi_{\alpha_1} \vee .. \vee \xi_{\alpha_K}) \Big), \end{aligned} \tag{3}$$

with

$$\xi_{\alpha_j} = \begin{cases} x_j & \text{if element } \alpha, j \text{ of truth table} = 0 \\ \neg x_j & \text{if element } \alpha, j \text{ of truth table} = 1 \end{cases},$$

having exactly 2^K clauses of $K+1$ elements. Thus, a network with connectivity K maps into a $(K+1)$-Sat having always $\alpha = 2^K \gamma$. However, we cannot imply directly that the typical behavior, and therefore the phase diagram of the system will be the same as that of the corresponding random

k-Sat. Considering random realizations of the constraints, these are *a priori* only a subset of all the possible realizations of a k-Sat constraint. In fact, it is immediate to realize that the 2^K CNF clauses written in Eq. (3) contain all the possible (fixed) combinations for the inputs variables and the corresponding (random) 2^K outputs for the output variable. This is best exemplified on the factor graph (Fig. 4).

Having established a link between GR1 and a particular optimization problem we set out to look at random instances for the signal integration functions. For fixed connectivity one can expect a similar behavior for K-GR1 as random k-Sat, or k-XORSAT (a Sat problem with clauses containing only XOR clauses), with the presence of a phase transition in the number of satisfying states. The suitable order parameter for such a transition is $\gamma = M/N$. Notably, the space of functions of the three models have different dimensions. Furthermore, differently from Sat or XORSAT, in GR1 each gene expression variable is regulated at most by one signal integration function, so that $\gamma \leq 1$. In practice, both for ease of interpretation and for simplicity of the analytical formulation, from now on we will abandon the formulation of GR1 in terms of CNF constraints, and work with the input-output functions f_n.

V. LEAF REMOVAL AND THE COMPUTATIONAL CORE

The aim of this section is to compute the number of satisfying solutions $\mathcal{N}$, for random instances of the constraints. For accessory reasons, we will map GR1 to a spin system. Quite simply, each Boolean variable $x_i \in \{0, 1\}$ is transformed into a spin $\sigma_i \in \{-1, 1\}$. Each constraint, or diamond function node generates the interaction Hamiltonian $H_{\diamond,b}$

$$2^k H_{\diamond,b} = \sum_{J_{b_1},..,J_{b_K}} \prod_{l=1..K} (1 + J_{b_l}\sigma_{b_l})(1 + J_{b_0}^{\{J_{b_1},..,J_{b_K}\}}\sigma_{b_0}) \,, \tag{4}$$

Under the restrictions defining GR1, the total energy of the system is simply the number of violated constraints. A zero-energy configuration satisfies all the constraints, and is therefore able to comply to all the logic functions encoded by the network. The 2^K coupling constants $J_{b_0}^{\{J_{b_1},..,J_{b_K}\}} = \pm 1$ are a representation of the truth table of the function f_b. With the correspondence $\{0, 1\} \leftrightarrow \{-1, 1\}$, $J_{b_1}, .., J_{b_K}$ stand for the possible values of the input variables $x_{b_1}, .., x_{b_K}$, while $J_{b_0}^{\{J_{b_1},..,J_{b_K}\}}$ is the associated output value. The Hamiltonian (4) is the cost function of the corresponding optimization problem K-GR1. It encodes the logic constraint of Eq. (2), in the sense that it is one whenever the constraint is violated, and zero otherwise. From the point of view

of transcription networks, $H_{\diamond,b}$ is the coarse grained local free energy of the Shea-Ackers model. Note that, even in the case of Boolean variables, the coupling constants represent binding affinities and interactions between transcription factors. In general, they need not be plus or minus one. Here we took this further assumption.

The conventional average of $\mathcal{N}$ on the realizations might be biased by the weight of exceptions (Mezard et al., 1987). The correct quantity to compute is the "quenched average" of the system's free energy, $\overline{\log \mathcal{N}}$, which is usually accessed with the replica, or similar methods (Mezard et al., 2002), passing from Hamiltonians like $H_{\diamond,b}$. However, in the case under examination we will use a simpler method, based on the "leaf removal" (Mezard et al., 2003) algorithm, which allows to compute only the *annealed* average $\log \overline{\mathcal{N}}$. As we will discuss, this method has the advantage of an immediate biological interpretation in terms of the roles played by genes in the network. For the case of random XORSAT, Mezard and collaborators (Mezard et al., 2003) have shown that the annealed average on the core variables coincides with the quenched one. In general $\overline{\log \mathcal{N}} \leq \log \overline{\mathcal{N}}$. For GR1, we performed estimates that indicate the presence of the same self-averaging property in a well-defined region of parameter-space (see sec. VI). Within this region, our annealed calculation is exact.

For a given realization of the constraints $\{\vec{I}, \vec{f}\}$, the number of satisfying states $\mathcal{N}$ can be written as

$$\mathcal{N}(\vec{I}, \vec{f}) = \sum_{\vec{\sigma}} \prod_{b=1}^{M} \delta(1; f_b(\sigma_{i(b,1)}, .., \sigma_{i(b,K_b)})\sigma_{i(b,0)}).$$

Here, the randomness is contained: (i) in the specification of the network structure, $\vec{I} = (I_1, ..., I_M)$, i.e. in the coordinates $i(b, l)$, which point at the variable occupying place l in the bth constraint; (ii) in the specification of the functions $\vec{f} = (f_1, ..., f_M)$ with a certain probability distribution in the class $\mathcal{F}$. An overbar ($^-$) indicates an average on both distributions, $p(\vec{I})$ and $p(\vec{f})$. We will first concentrate on the case with fixed in-ward connectivity K.

In carrying this average, there are three relevant preambles. The first is that not all the M equations and N variables are meaningful to calculate $\mathcal{N}$. Indeed, every output variable that appears in only one constraint can be trivially fixed according to its function. Thus, both the constraint and the variable can be eliminated without affecting the number of solutions. This procedure is called "leaf removal" (Mezard et al., 2003). It is a nonlinear procedure, as more variables can disappear together with a single constraint, because input free genes that regulate a leaf remain as isolated points. The iteration of this mechanism leads to the definition of a "core", the CCC, of signifi-

cant variables and constraints, in numbers of N_C and M_C respectively. In the CCC, M_C genes are controlled, and $\Delta_C = N_C - M_C$ are the "free" genes with an essential role in controlling the expression states, as a function of an input signal. The second relevant fact is the hypothesis that the functions are independent and identically distributed random variables. Thirdly, we consider a set of functions in a family $\mathcal{F}_K$ which satisfy the condition $\frac{1}{2^{2^K}} \sum_{f \in \mathcal{F}_K} p(\vec{f}) f(\vec{x}) = \rho$, as will always be the case if the outputs of the functions are uncorrelated, even in the presence of bias.

It is then easy to verify that

$$\overline{\mathcal{N}} = \sum_{\vec{\sigma},\vec{I}} p(\vec{I}) \prod_{b=1}^{M} \left(\rho \delta(1; \sigma_{i(b,0)}) + (1-\rho) \delta(-1; \sigma_{i(b,0)}) \right) \; ;$$

so that, as for the XORSAT model,

$$\overline{\mathcal{N}} = 2^{N_C - M_C} \; . \tag{5}$$

Incidentally, we note that the same procedure is valid for GRq, where $\overline{\mathcal{N}} = q^{N_C - M_C}$.

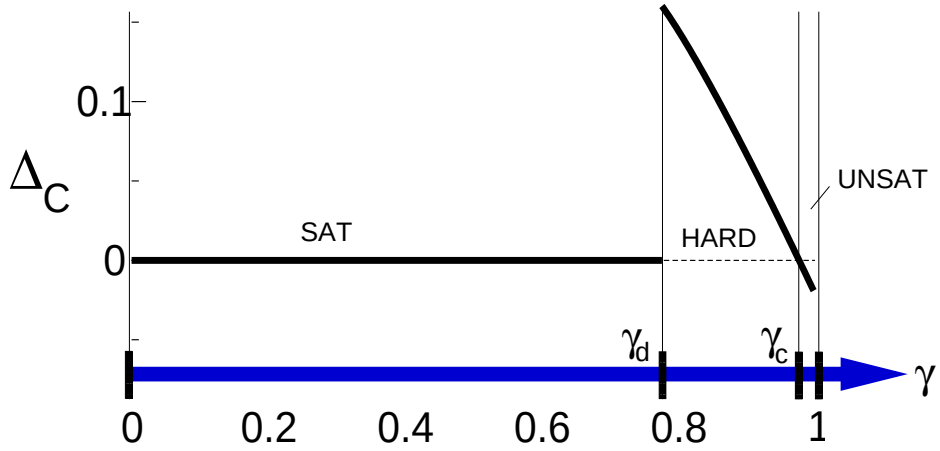


FIG. 5 Phase diagram of 4-GR1. For $\gamma > \gamma_c$ no solutions exist in the typical realization (UNSAT phase). For $\gamma < \gamma_d$ the system is paramagnetic typically a satisfying state exists (SAT phase, or simple gene control). For $\gamma_d < \gamma < \gamma_c$ there is complex gene control.

We can use $\frac{\Delta_C}{N_C}$ as an order parameter in the thermodynamic limit $N \to \infty$, γ const., to distinguish three types of phenomenology, or three "phases". (i) For $\frac{\Delta_C}{N_C} \leq 0$, there are no free genes in the core, and the system cannot comply to all the expression programs encoded into its DNA. This is a UNSAT phase from the computational point of view. (ii) For $0 \leq \frac{\Delta_C}{N_C} < 1$, the Δ_C genes of the CCC control $O(N)$ genes each, and therefore are able to determine an expression state. This is a "complex gene control" phase, or HARD-SAT (iii) For $\frac{\Delta_C}{N_C} = 1$ the number of controlled variables is a vanishing fraction of the total number of genes. In other words, $M_C = 0$ in the thermodynamic

limit, the free genes control at most $O(1)$ genes to generate a satisfying state ("simple control", or SAT phase). In the simple control phase, the system is underconstrained, which means that the logic conditions imposed by the signal integration functions are generally insufficient for a strict determination of the expression patterns.

The three phases described above depend both on the value of γ and on the class of random functions considered. In general, if all the possible functions are taken into account, the phase diagram can be explored studying the rank and the kernel of the connectivity matrix (Caracciolo and Sportiello, 2002). Following the analysis of Mezard and collaborators (Mezard et al., 2003), in the case of Poisson variable connectivity, i.e. with the distribution $\pi(c) = \frac{(k\gamma)^c}{c!}e^{-k\gamma}$, the phase diagram as a function of γ is identical to the random XORSAT problem. It is illustrated in Fig. 5. For $\gamma > \gamma_c$ no solutions exist in the typical realization (UNSAT phase). For $\gamma < \gamma_d$ the system is paramagnetic (SAT phase). For $\gamma_d < \gamma < \gamma_c$ exponentially many satisfying states exist. Here, the space of solutions breaks down into clusters separated by free energy barriers. The typical dynamics in a cluster will be residual, in the sense that a block of genes are fixed (on or off) and the rest may move. The number of clusters is controlled by the (computational) complexity Σ of the system. The number of observed configurations is $\mathcal{N}^* \sim \exp[N\Delta_C]$. Thus, by definition Σ is directly related to the order parameter Δ_C/N_C, i.e. to the partitioning of the core genes. How the system explores (or not) the clusters depends on details of its dynamics.

VI. STATE-FLUCTUATIONS AND SELF-AVERAGE

In this section, we discuss a calculation of the *width* of the distribution of the number of compatible states. In order to do this, we compute the quantity $\overline{[\mathcal{N}]^2}$. This calculation is relevant both from the technical and from the qualitative point of view. The technical aspect, as already anticipated, deals with the self-averaging property, which holds when the quantity

$$\frac{\overline{[\mathcal{N}]^2} - \overline{[\mathcal{N}]}^2}{\overline{[\mathcal{N}]}^2}$$

vanishes in the thermodynamic limit $N \to \infty$ at constant γ. When this condition is met, the annealed average computed above coincides with the quenched one, and no extra effort is required. When it is not, the behavior of the system can be qualitatively different from what emerges in the annealed picture. In particular, the typical number of solution is overestimated. In this case, more complicated formalisms, such as replicas, need to be adopted (Mezard et al., 1987).

The qualitative aspect involves the possible physical, and biological, interpretation of $\overline{[\mathcal{N}]^2}$. This is an indicator of the width of the probability distribution in the number of compatible states, in presence of random functions and network structure. Therefore, it can be seen as the freedom the system has of varying the number of states that comply to the signal integration functions by acting on its constraints. Biologically, having in mind Darwinian evolution one may interpret it as a kind of "adaptability". This is done as follows. If $\mathcal{N}$ is interpreted as the number of possible responses of gene patterns to external or internal conditions, it is reasonable to assume that a given system with a fixed number of genes will be fitter by maximizing $\mathcal{N}$. Now, if the distribution is wide, the system can vary greatly $\mathcal{N}$ by acting on the signal integration functions. If the distribution is peaked, the changes in the functions will be less effective in increasing the number of states.

If the self-averaging property holds, all the width, this adaptability, comes only as a finite-size effect. This is not irrelevant, considering that the value of N is in the range $10^3 - 10^5$, quite far from the usual Avogadro's number! On the other hand, if the self-averaging property does not hold, it means that a residual width exists even in the thermodynamic limit. In this case, an evolved system can be ultra-specific, finding the very exceptional situations in which a high number of solutions exists against the typical odds in which the system cannot express compatible gene patterns, the biblical needle in a haystack. Such putative highly specialized organisms would be particularly sensitive to changes in the environment. Given these considerations, it seems that the lack of self-averaging for a model like GR1 would make it slightly less appealing.

The final result for GR1 is that $\mathcal{N}$ is indeed self-averaging. The details of the calculation are reported in Appendix A. It relies on two basic assumptions. The first relates to the choice of a family of random functions such that:

(a) $\frac{1}{\Lambda}\sum_{f\in\mathcal{F}} p(\vec{f})f(\vec{\sigma}) = 0$, as above, and

(b) $\frac{1}{\Lambda}\sum_{f\in\mathcal{F}} p(\vec{f})f(\vec{\sigma})f(\vec{\tau}) = \delta(\vec{\sigma};\vec{\tau})$.

Here, Λ indicates the size of the family of functions, and we used the obvious notation that makes the functions assume values ± 1 if expressed in terms of spins.

The second assumption in the computation can be seen as a mean-field-like hypothesis of independence of spins belonging to different clauses. It can be argued as follows. Differently from the evaluation of $\overline{\mathcal{N}}$, the computation of $\overline{[\mathcal{N}]^2}$ depends both on the class of functions and on the underlying network. The essential problem is that while the function nodes are independent random variables, the variable nodes clustered by functions can be repeated. Thus, one has to answer to the question: how many distinct variables n_v are connected to r constraints? For small r, we

can estimate that $n_v \simeq r \cdot k$, while for $r \sim M,\ n_v \simeq \frac{M}{\gamma}$. These extremes set the "fork" of values for which our estimate is consistent and robust. In these conditions, we obtain the scaling as 4^{Δ_C}, in the relevant regime of the phase diagram $1/K < \gamma < \gamma^*$, with $\gamma^* > \gamma_c$. This enforces the self-averaging property. The same procedure can be carried with the k-Sat model yielding no self-averaging for the number of satisfying states.

VII. DIFFERENT CONNECTIVITIES

So far we have discussed the idealized case where the in-ward connectivity, the number of transcription factors controlling one gene, is fixed. In that case, only the out-ward connectivity, or the number of genes controlled by a transcription factor, can fluctuate. A biologically more realistic case is when both the inward connectivity K and the outward connectivity c vary along the network, and the decay of the latter is slower (see Fig. 8a).

Considering $p(k|c) = \frac{(k\gamma)^c}{c!}e^{-(k\gamma)}$, the conditioned probability that a variable is in c clauses of the k kind, we have $\pi(c) = \sum_k \frac{(k\gamma)^c}{c!}e^{-(k\gamma)} \cdot p(k)$. The leaf removal algorithm can be applied separately to sets of clauses with a given connectivity, defining $N_C \equiv< N_C >_k$ and $M_C \equiv< M_C >_k$, where $< X >_k = \sum_p p(k) \cdot X(k)$. Choosing $p(k) = Z^{-1}(\nu)e^{-\nu k}$ does not affect the exponential asymptotic decay of $\pi(c)$ for large c.

To show this, let us construct the graph with the mentioned properties

1. The probability to have a clause with k elements is $p(k) = Z^{-1}(\nu)e^{-\nu k} \quad (k > 1)$.
2. $p(k|c) = \frac{(k\gamma)^c}{c!}e^{-(k\gamma)}$ is the conditioned probability that a variable is in c clauses of the k kind.
3. $\pi(c) = \sum_k \frac{(k\gamma)^c}{c!}e^{-(k\gamma)} \cdot p(k)$.

and compute $\pi(c)$. Setting $\xi = \gamma + \nu$,

$$\pi(c) = \frac{\gamma^c}{c!}\left(-e^{-\xi} + \mathcal{Z}[k^c]\right) \quad ,$$

where $\mathcal{Z}[f(c)] = \sum \frac{f(c)}{z^c}$ is the Z-transform, and for us $z = e^{\xi}$. Now, $\mathcal{Z}[p^c] = \mathrm{Li}_{-c}(z^{-1})$, where Li is the polylogarithm, which can be defined for negative integers as

$$\mathrm{Li}_{-c}(z) := \frac{1}{(1-z)^{c+1}} \sum \left\langle {c \atop i} \right\rangle r^{c-i} \quad ,$$

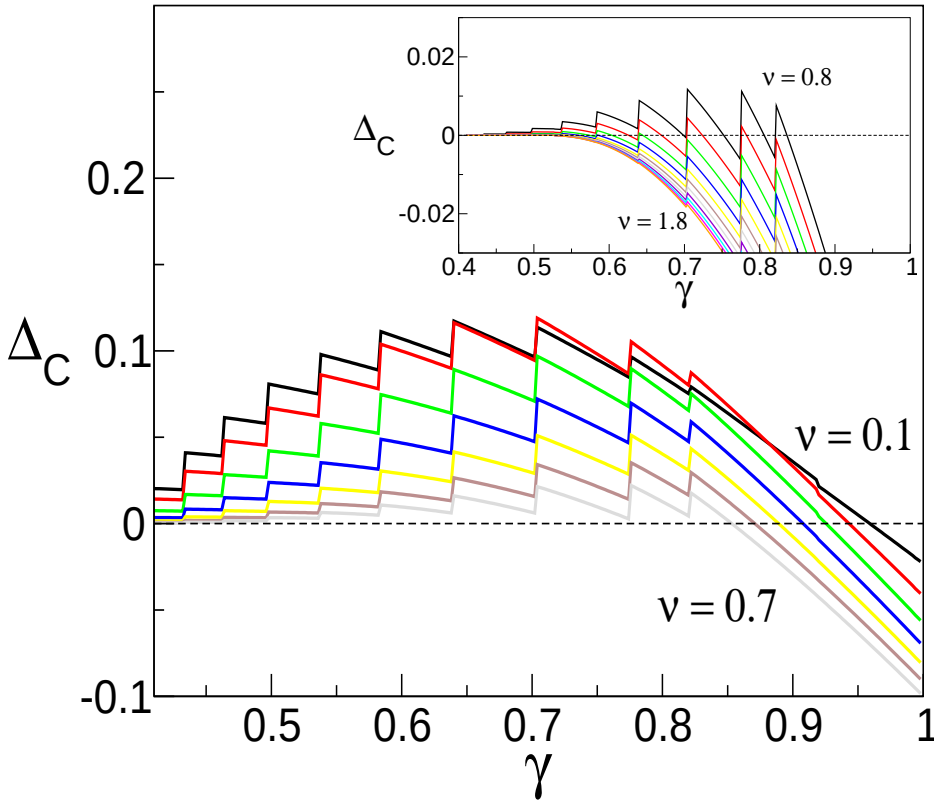


FIG. 6 Δ_C as a function of γ for different values of ν in the multi-Poisson case. The discrete jumps are due to the onset of HARD phases for the different values of k. Δ_C can become negative many times, giving rise to reentrant UNSAT phases. The figure refers to a connectivity distribution with a cutoff at $k = 12$

where $\left\langle {c \atop i} \right\rangle$ are Euler's numbers. This gives a condensed expression for $\pi(c)$:

$$\pi(c) = \frac{\gamma^c}{Zc!} \left\{ \mathrm{Li}_{-c}(e^{-\xi}) - e^{-\xi} \right\} \quad .$$

It can be easily checked that, for large c, this function decays exponentially, after having reached a maximum.

We can call this case multi-Poisson, as the graph is a superimposition of graphs that follows a Poisson distribution, each graph having in turn a fixed clause-connectivity and a Poisson variable-connectivity. The behavior of GR1 on such a topology is different from the fixed connectivity case. The main reason for this is that, while $\Delta_C(\gamma)$ is still locally decreasing, many new discontinuities emerge, due to the influence of clauses with different connectivities. This gives rise to two phenomena. Firstly, Δ_C can increase globally with increasing γ. Indeed, it does increase after γ_d, to decrease again before γ_c (Fig. 6). After the onset of the complex control phase, the complexity initially increases (step-wise), reaches a maximum, and then decreases monotonically. This has an influence on the number of observed states as a function of γ. Secondly, Δ_C can become negative

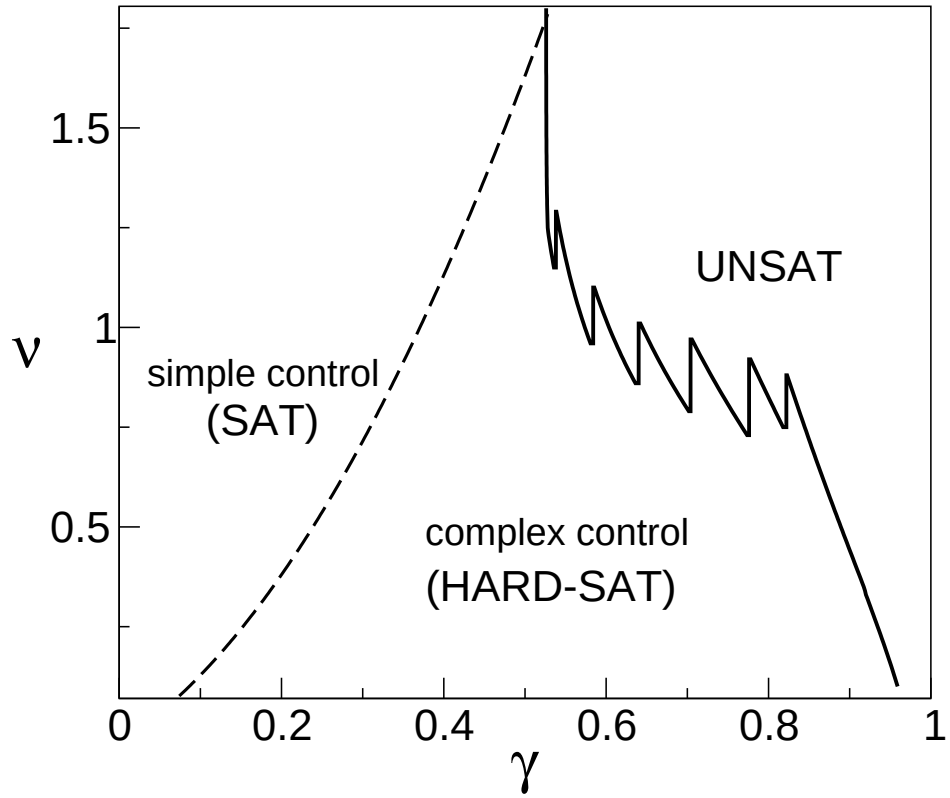


FIG. 7 Phase diagram $\gamma - \nu$ for the multi-Poisson case. The dashed line represents the mean value of the numerically evaluated critical parameter $\gamma_d(\nu)$ for the SAT-HARD transition of network realizations with $N = 3 \times 10^4$.

and then jump back to a positive state, creating a reentrant HARD-SAT phase (Fig. 7). We are currently studying ways to extend our calculation of the mean number of compatible states and the width of its distribution on graphs with more general connectivities.

VIII. AN EXAMPLE FROM AN EXPERIMENTAL SETTING

The results described so far focused on the typical behavior of GR1 as a formal model for a genetic network. To resume them, we can predict the existence of a core of variables, the CCC, which determines the behavior of the system. The phase diagram of the system contains two regimes of gene control, simple and complex. In the complex control phase, the free genes of the core control $O(N)$ other genes. These phases also depend on connectivity. On the other hand, a very important question is how to relate them to concrete systems. There are many possibilities in this direction that we are currently exploring. In this section, we will discuss a first attempt. Specifically, we will make use of the data set for the structure of the E. coli transcription network

from the RegulonDB database (Salgado et al., 2001), with the modifications of (Shen-Orr et al., 2002). The goal is to apply the leaf removal algorithm using the information contained in the data set.

The data set consists of an annotated graph, where the signal integration functions are described as sets of annotated links. The annotations consist in three modes of activity: activation, repression, and "dual" activity (meaning that the activity depends on the context). The data on the combinatorial activity of transcription factors are not part of the set. For this reason, in what follows we will ignore the annotations, concentrating on the study of random GR1 realizations on the given experimental network structures. Considering the connectivity matrix C_{ij} defined as

$$C_{ij} = \begin{cases} 1; & \text{Gene j regulates gene i} \\ 0; & \text{Otherwise} \end{cases} ,$$

a "leaf" corresponds to a column containing only zeros. An iteration of the algorithm removes these columns, together with the corresponding lines. Note that the leaf removal algorithm is not guaranteed to preserve the network structure as in the abstract cases discussed above.

During the iterative leaf removal procedure, one is confronted with an important choice, concerning how to deal with autoregulators. These create a problem, as, for particular assignments of the functions they create trivial contradictions. In reality, this self-contradiction is inexistent, as negative autoregulations are known to play the role of controlling the overexpression of a particular gene. A standard, and the simplest, way to avoid the problem is simply to eliminate the autoregulations, and impose that the diagonal of C_{ij} is zero. However, this total cancellation is not biologically motivated - as autoregulations might reflect some global properties of the system, other than control of overexpression. To clarify, let us consider a gene that regulates itself and is regulated by some others ("rest"), that is a "regulated autoregulator" (RAR). It is then subject to the constraint $\sigma_0 = f(\sigma_0|\text{rest}) = A(\text{rest})\sigma_0 + B(\text{rest})$. If $A(\text{rest}) = 0$ the gene is regulated simply, for $B \in \{-1, 1\}$ and the autoregulation is irrelevant. Conversely, when $B(\text{rest}) = 0$, the autoregulation plays a role, but if $A(\text{rest}) = -1$ the system is UNSAT.

To solve this problem, we propose a way to keep the role of autoregulators into account, while at the same time avoiding the trivial self-contradiction. In order to do this, we introduce the constraint $A(\text{rest}) = 1$ that codes for the avoidance of trivial contradictions. With this technique, we aim to save the autoregulation role, while taking into account the notorious fact that autoinhibitions cannot be represented with Boolean variables. We can call this the "RAR hypothesis".

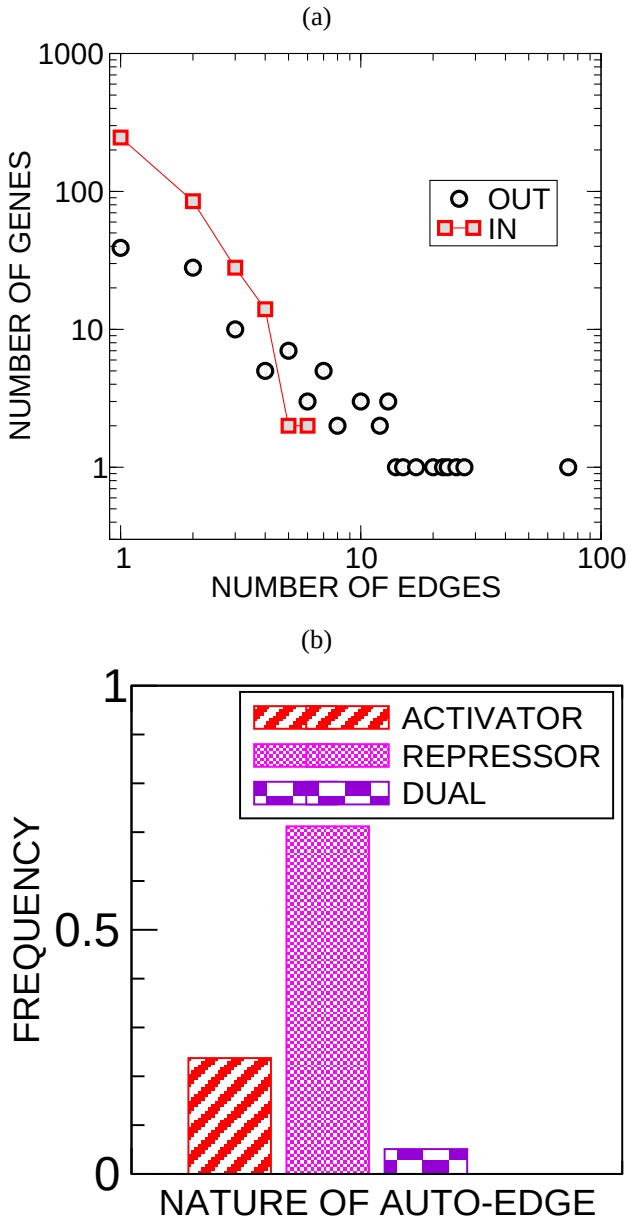


FIG. 8 Data inferred from the E. coli transcription network. (a) Degree distribution. (b) Activity of autoregulators. See also (Martinez-Antonio and Collado-Vides, 2003)

.

We will see that this hypothesis brings to a different final result. The same reasoning can be carried for GRq-type variables. Assuming the RAR hypothesis, the problem becomes a mixed optimization problem, that includes the usual GR1 constraints, plus a set of Sat-like constraints that come from the $A_n(\text{rest}) = 1$ conditions on the RARs.

A. The E. coli Transcription Network

In the E .coli data set there are 423 genes, and 59 autoregulations. Among these, 24 are RARs (Fig. 8). Applying the leaf-removal algorithm with cancellation of autoregulations leads to an empty core. This means that the system finds itself in the simple control, SAT phase. However, the application of the RAR hypothesis leads to a non-empty core (Fig. 9).

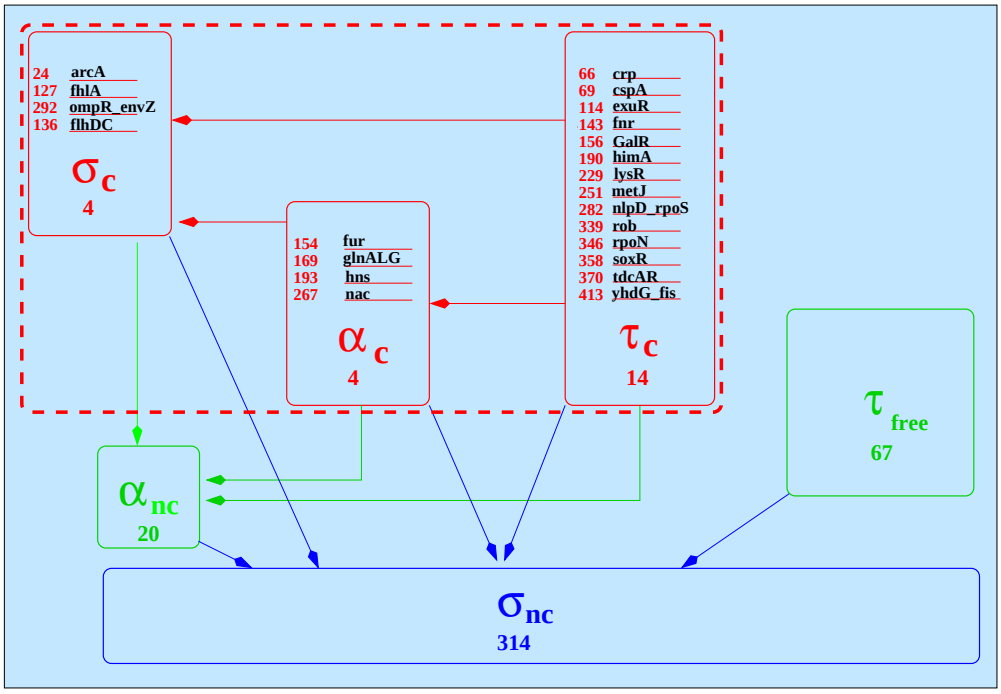


FIG. 9 The CCC of E. coli with the RAR hypothesis. It contains 22 variables (of which 14 are free or regulated only by themselves, 4 are non-free, and 4 are RARs) and 22 constraints (of which 18 are RAR constraints).

The genes in the core can be divided in three different classes, free, which we will denote by τ, non-free (σ), and RARs, or (α). The core contains a total of 22 variables (of which 14 are free or regulated only by themselves, 4 are non-free, and 4 are RARs) and 22 constraints (of which 18 are RAR constraints). Biologically speaking, these core gene include some “global regulators”, or transcription factors with a high out-ward connectivity (Martinez-Antonio and Collado-Vides, 2003), including (a) the sigma factors rpoS and rpoN, (b) proteins belonging to the family of the DNA bending global regulator crp (c) himA, or IHF, another DNA bending factor. More interestingly, also lower connectivity proteins, connected to metabolism (e.g. respiratory control and iron transport), and to structural tasks (e.g synthesis of the flagellum) are found in the core.

The residual optimization problem on the core variables is small and simple enough to be

solved for general functions, as exemplified in Fig. 10. The final solution gives only two states, after having fixed the free genes.

What is the meaning of the CCC in the RAR hypothesis, if any? The answer can come from two directions: simulations and experiments. For the numerical case, one must study how fixing the core variables affects the reach of a fixed point or a steady state in a Boolean network. Our simulations on both asynchronous spin-flip and synchronous update dynamics show that, fixed some random functions on the whole network, the core free genes control a larger set of genes than the non-core ones (these results will be published elsewhere (Cosentino Lagomarsino et al., 2005)). This is an indication that the CCC found with the RAR hypothesis might have some significance. The same feature can be tested with microarray expression experiments.

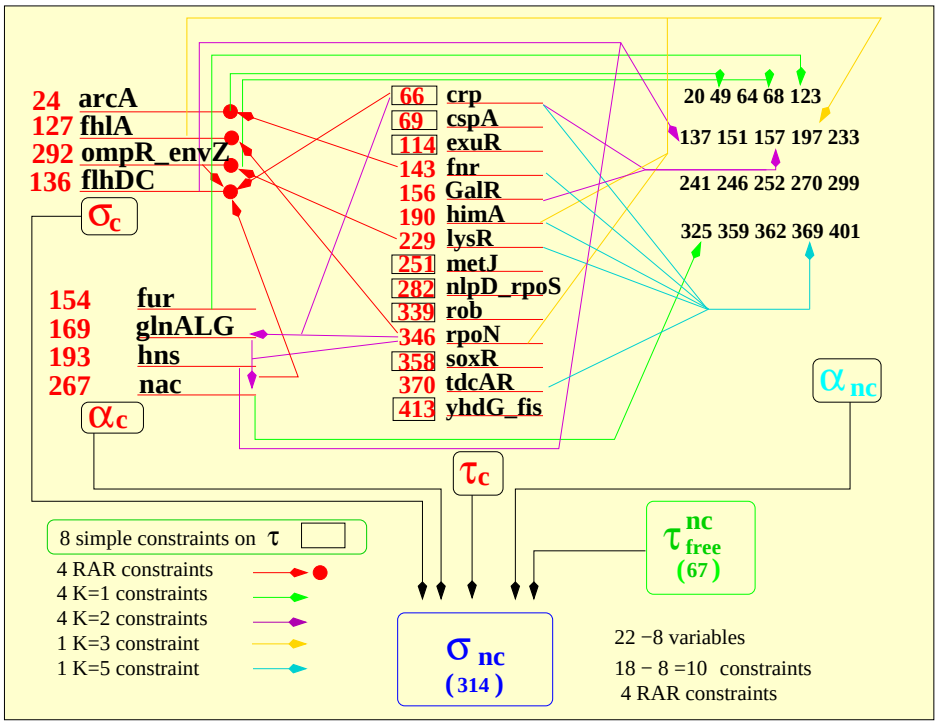
IX. CONCLUSIONS

In conclusion, we have presented and discussed a novel conceptual framework for the equilibrium modeling of large scale transcription networks. In its most general formulation, our approach is directly connected to the Shea-Ackers model for the *cis*- regulatory region of a gene, and consists of a compatibility analysis of the constraints established by the signal integration functions.

The advantage of this approach is that it allows to separate issues related to the dynamics of the network from the basic logic structure that underlies it. Obviously, dynamics is a very important factor of a real biochemical network, possibly the most important. On the other hand, we feel that the disentanglement the two aspects might lead to further insight. In the spirit of theoretical computer science, any dynamics superimposed on GR1 can be seen as an algorithm. The problem becomes then the following. How effectively does a given algorithm, modelling chemical kinetics explore configuration space? Naturally, this addition may carry intricate issues, connected to the nonequilibrium nature and the asymmetry of the interactions. These issues are particularly complex if one wants to add a coarse-graining of time, as is commonly done in Kauffman networks. In absence of an explicit knowledge of the emergent time scales involved in the dynamics, we feel ours is an appropriate approach. Particularly in the Boolean approximation, GR1, which we treat here.

From a general, speculative standpoint, our model shows that the "biological complexity" is not simply measured by the number of genes. For a transcription network, a more proper indicator is Δ_C. Interestingly, for GR1 this coincides exactly with what is called the "computational"

(a)



(b)

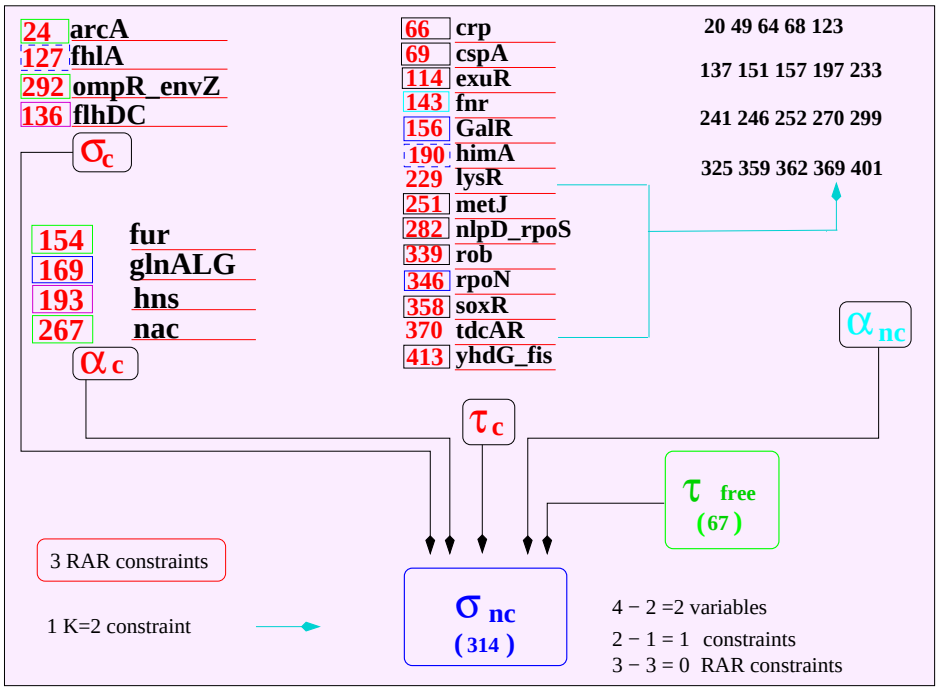


FIG. 10 Solution of the general optimization problem on the core variables of E. coli in the RAR hypothesis. In this procedure, variables are fixed with respect to each other according to the constraints that connect them. (a) Second step of the computation (b) Last step of the computation.

complexity, Σ. Looking at the phase digram, Σ depends on the order parameter γ, or - loosely - on the number of transcription factors per gene. At fixed number of genes, it is known that this quantity increases in bacteria that need to react to more environments (Cases et al., 2003). Imagining that prokaryotes, being unicellular, naturally find themselves in a simple control phase, our phase diagram predicts an intrinsic limit to this adaptation process, represented by the phase boundary with the HARD-SAT. Considering varying N, one may wonder why, in real organisms, a small γ is correlated with a small N (van Nimwegen, 2003). A possible answer to this question is the following. With large N and small γ, the system is shifted to the SAT phase, and therefore needs to explore a very big configuration space without sufficient "guidelines". In other words, the available configurations are too many to be reached in reasonable time by the dynamics.

Similar considerations can be carried for the *width* of the distribution of satisfying solutions. The fact that the self-averaging property holds indicate that this is negligible in the thermodynamic limit. On the other hand, the typical value of N for a living system is in the range $10^3 - 10^5$. While being large for detailed modeling, this is a smaller number than the size of the typical system treated with statistical mechanics. Thus, the effects of the system size are expected to be important.

Considering the phase diagram in Fig. 5, the complex control phase, having general residual dynamics, matches a qualitative feature of many cells, where some genes are constantly expressed, and the rest vary. On the other hand, the dynamical slowing down characteristic of any glassy phase raises an issue that must be solved by the chemical dynamics of the cell. In analogy with Kauffman's ideas, the breakdown in many different attraction basins might be interpreted as epigenesis. That is, in the HARD-SAT phase there will be typically many cell types. How many, is determined by the complexity Σ, which is directly measured by our Δ_C. While for fixed K this quantity simply decreases with increasing γ, its behavior is more interesting in the multi-Poisson case. However, this remains an open issue which has to be regarded with more detail. The experimental scaling of the number of cell types is sub-linear in the number of genes N (Kauffman, 1993, 2004). In the fixed K case, GR1 gives exponential scaling with N at fixed γ in the complex control phase. On the other hand, the results of random-GR1 are perhaps more easily related to the number of species times the number of cell types at equal number of genes. In the same way, the behavior of $\overline{[\mathcal{N}]^2}$ with N should roughly predict the scaling of the *variability* in the total number of cell types for all the species with equal number of genes N. According to GR1, this quantity should vanish in the thermodynamic limit.

To be biologically useful, the model has to deal with the details of an individual realization the system. In this respect, an advantage of the leaf removal algorithm is that it transforms a problem related the states of variables on a graph, the gene expression patterns, into a problem regarding the *structure* of the graph. This is particularly of interest as long as the data regarding the activity of function nodes are only partially known. For example, the first application to the E. coli core, in the RAR hypothesis leads to interesting results, that have a numerical counterpart and might be tested with expression correlation data. The application to more, larger, data sets and to other forms of regulation might lead to further insight. Notably, some of the core variables do not have a high connectivity. This is an indication that additional, global, properties of the network structure other than local order parameters must contribute to establish the hierarchy of states in configuration space.

Finally, besides the extensions to the work presented here, we believe the framework of GR might be used as a setting for many different problems involving fairly large networks, from evolutionary models to regulation network optimization, from network inference to design. It will be potentially useful in the years to come, as more and more data will be available from high-throughput experiments.

Acknowledgments

We would like to acknowledge interesting discussions with L. Finzi, A. Sportiello, J. Berg, M. Leone, M. Caselle, P.R. ten Wolde, R. Zecchina.

APPENDIX A: Self-averaging property of GR1.

The following paragraphs describe the calculation of the width of the distribution of $\mathcal{N}$. By definition,

$$\overline{[\mathcal{N}]^2} = \sum_{\vec{\sigma},\vec{\tau}} \sum_{C} p(C) \sum_{f^1 \in \mathcal{F}} p(f^1) \sum_{f^1 \in \mathcal{F}} p(f^2) ... \sum_{f^M \in \mathcal{F}} p(f^M) \cdot$$

$$\cdot \prod_{m=1}^{M} \delta\big(1 - f^m(\sigma_{n(1,m)}, \cdots, \sigma_{n(k,m)}) \cdot \sigma_{n(0,m)}\big) \delta\big(1 - f^m(\tau_{n(1,m)}, \cdots, \tau_{n(k,m)}) \cdot \tau_{n(0,m)}\big) \ ;$$

thus,

$$\overline{[\mathcal{N}]^2} = \sum_{\vec{\sigma},\vec{\tau}} \sum_{C} p(C) \prod_{m=1}^{M} \Big(\sum_{f^m \in \mathcal{F}} p(f^m) \delta\big(1 - f^m(\sigma) \cdot \sigma_{n(0,m)}\big) \delta\big(1 - f^m(\tau) \cdot \tau_{n(0,m)}\big) \Big) \ .$$

For fixed states $\vec{\sigma}$ and $\vec{\tau}$, we can write the factors of the product above as

$$\Big(\delta\big(1-\sigma_{n(0,m)}\big)\delta\big(1-\tau_{n(0,m)}\big)\cdot A(\sigma_m;\tau_m)+\delta\big(1-\sigma_{n(0,m)}\big)\delta\big(1+\tau_{n(0,m)}\big)\cdot B(\sigma_m;\tau_m)+$$

$$\delta\big(1+\sigma_{n(0,m)}\big)\delta\big(1-\tau_{n(0,m)}\big)\cdot C(\sigma_m;\tau_m)+\delta\big(1+\sigma_{n(0,m)}\big)\delta\big(1+\tau_{n(0,m)}\big)\cdot D(\sigma_m;\tau_m)\Big)\ ,$$

Where A, B, C, D, are the weights of the functions $f\in\mathcal{F}$ such that, respectively

$$\begin{cases} A(\sigma_m;\tau_m) \ \leftarrow\ f(\sigma\in m)=1 \quad \& \ f(\tau\in m)=1 \\ B(\sigma_m;\tau_m) \ \leftarrow\ f(\sigma\in m)=1 \quad \& \ f(\tau\in m)=-1 \\ C(\sigma_m;\tau_m) \ \leftarrow\ f(\sigma\in m)=-1 \ \& \ f(\tau\in m)=1 \\ D(\sigma_m;\tau_m) \ \leftarrow\ f(\sigma\in m)=-1 \ \& \ f(\tau\in m)=-1 \end{cases}$$

Now, as $A+B+C+D=1$, and $A+B=A+C=\rho$, we can write, choosing $\rho=1/2$

$$\sum_C\sum_{\vec{\sigma}\vec{\tau}}\prod_m$$

$$\{\ A(\sigma_m,\tau_m)\cdot\big[\delta\big(1-\sigma_{n(0,m)}\big)\delta\big(1-\tau_{n(0,m)}\big)-\delta\big(1+\sigma_{n(0,m)}\big)\delta\big(1+\tau_{n(0,m)}\big)\big] \ + \quad (\mathcal{A})$$

$$+\frac{1}{2}\big[\delta\big(1-\sigma_{n(0,m)}\big)\big(1+\tau_{n(0,m)}\big)+\delta\big(1+\sigma_{n(0,m)}\big)\big(1-\tau_{n(0,m)}\big)\big]\ \} \quad (\mathcal{R})$$

The product on m gives rise to 2^M terms of the kind

$$\prod_{k=1}^{r}\mathcal{A}^{(k)}\prod_{k'=r+1}^{M}\mathcal{R}^{(k')}\ ,$$

where $\mathcal{A}$ and $\mathcal{R}$ indicate factors of the two types in the previous expression. For every r there are $\binom{M}{r}$ terms of this kind in the sum. Applying the properties that characterize our family of functions, we find $A=\sum_{f\in\mathcal{F}}\big[\big(\frac{1+f(\sigma)}{2}\big)\big(\frac{1+f(\tau)}{2}\big)\big]=\frac{1}{4}\big[1+\delta(\sigma,\tau)\big]$

With this consderation, the sum over the configurations $\vec{\sigma}\vec{\tau}$ can be simplified. It involves the product

$$\prod_m$$

$$\{\ A(\sigma,\tau)\cdot\big[\sigma_{n(0,m)}\tau_{n(0,m)}\big] \ + \qquad (\mathcal{A})$$

$$+\frac{1}{4}\big[1-\sigma_{n(0,m)}\tau_{n(0,m)}\big]\ \} \qquad (\mathcal{R})$$

Let us now distinguish again between free genes and non-free ones, which are outputs of some signal integration function. The sum over non-free genes is such that (i) there is a contribution $\frac{1}{2}^r$ due to the Kronecker deltas in the $\mathcal{A}$ part and (ii) if a type $\mathcal{R}$ non-free $\sigma_{n(0,k')}$ or $\tau_{n(0,k')}$ variable

appears in the input of a type $\mathcal{A}$ clause the contribution is zero. A little thought leads to the conclusion that the non-free genes sum up to the term

$$\left(\frac{1}{2}\right)^r \left(1-\gamma+\frac{r}{N}\right)^{kr}$$

The sum over the $2(N-M)$ free genes would give a 4^Δ contribution in case of complete independence. However, a delta function on the input genes reduces the double sum to a single one. To estimate this contribution one has to evaluate the probability that two free genes appear as input of a type $\mathcal{A}$ clause. In a mean-field like estimate, this is $kr\frac{N-M}{N-M+r}$, leading to the contribution

$$4^\Delta \cdot 2^{-\frac{kr}{1+\frac{r}{\Delta}}}$$

The 4^Δ term factors out of everything, and alone would give the desired self-averaging property. It remains to evaluate the sum over r. Restricting the sum over the core genes, and evaluating it with a saddle point method leads to the minimization of the free-energy-like functional

$$G(x) = x\log x + (1-x)\log(1-x) + \log(2)x\left(1+\frac{k(1-\gamma)}{1-\gamma(x-1)}\right) - kx\log\left(1-\gamma(x-1)\right) \ ,$$

where $x \in [0,1]$, and $\gamma := \frac{M_C}{N_C}$. Minimization of this functional always leads to the solution $x=0$, with the exception of the regions: $\gamma < 1/K$ and $\gamma > \gamma^*$, where γ^* is a threshold that always lies in the UNSAT region. For $k=3$, $\gamma^* \simeq 0.9722$.

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